1

The machinery of signal transduction

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Molecular basis of signal transduction

In this chapter, the signals, the growth factors, and the receptors receiving the signals are introduced. Receptor–ligand interactions at the cell surface are the first step in cellular signalling. This introduction will acquaint the reader with some of the major players in cellular signalling.

Properties of receptors

Cellular signal transduction is a two-step process: First, a signalling molecule is sensed by a receptor at a target cell and then the receptor is activated (Fig. 1.1). Membrane-bound receptors respond to a large spectrum of extracellular signals. External signals range from light and odours to hormones, growth factors, and cytokines. When the receptor sensing the signal is a catalyst, a kinase, the response is amplified.[1] As diversified as the signals, are the proteins which respond to them. In each case, binding of a signalling molecule converts the dormant receptor to an active state. A mechanism involved in the transition of a receptor from its inactive to its active state is receptor oligomerization. This is discussed in Chapter 2.

All receptors that transmit signals from the surface of the cell to the interior, and finally to the nucleus and the genes, have two features in common:

(1) the signalling molecule binds to the extracellular domain of the membrane-inserted receptor; and

(2) ligand binding triggers, in a cooperative manner, a change in the domain inside the cell.

Although the molecular details of this conformational transition are not yet elucidated in all cases, it is to be expected that they differ as much as the structure of the receptor. For all membrane-bound receptors, the effects of the environment, the lipid bilayer, should be taken into account, although little is known about such influences.

Upon binding the ligand and activation, ligand–receptor complexes are eventually internalized. Internalized ligand–receptor complexes are dissociated in acidic

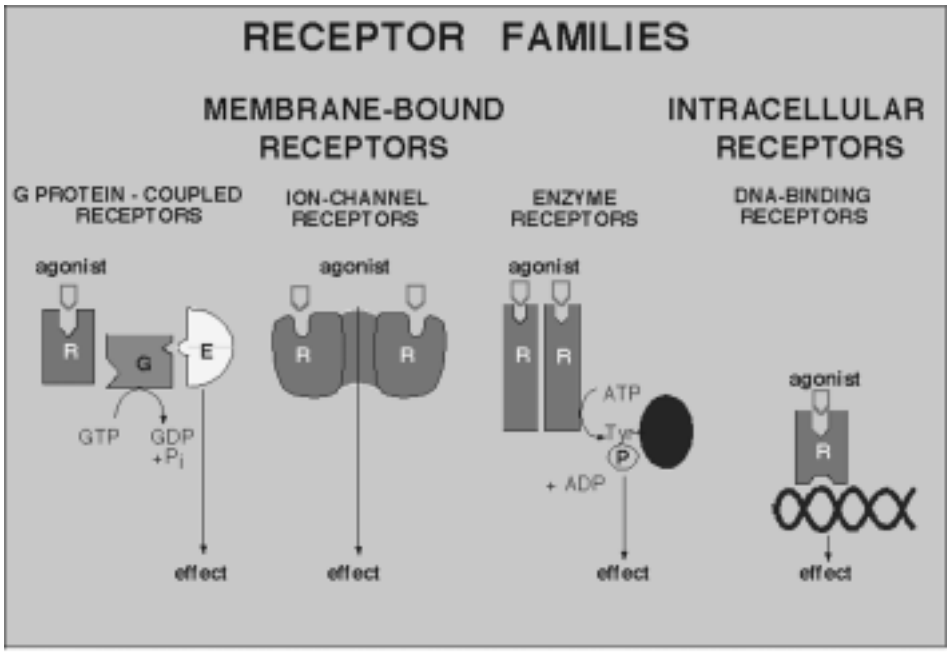

Fig. 1.1 Four major families of receptors are shown: From left to right: G-protein-coupled receptors. A representative example of this receptor family is rhodopsin, which is responsible for visual perception in the eye. R is the receptor; G is the GTP-binding protein; E is the effector. Effectors are often enzymes which form second messengers. An example is adenylyl cyclase, which catalyses the synthesis of cAMP from ATP. In the case of the visual response, the effector is a cGMP phosphohydrolase, which converts cGMP to GMP (see Chapter 5). A representative of ion-channel receptors is the nicotinic acetylcholine receptor. This type of receptor will not be discussed in this book. Enzyme receptors are transmembrane receptors with intrinsic enzymatic activity. Examples are the receptor tyrosine kinases (RTKs), which autophosphorylate their own tyrosine residues, such as the growth factor receptors and the insulin receptor. And, finally, there are the intracellular DNA-binding receptors. They bind lipophilic ligands that pass through the membrane. They address genes directly. Examples are the steroid hormone receptors (see Chapter 11). (This figure was donated by Professor Martin Lohse, University of Würzburg.)

endocytosolic vesicles and the ligand is degraded in lysosomes, whereas the receptor may be degraded or recycled back to the cell surface. Receptor–ligand complexes may be internalized together with proteins which regulate their endocytosis and degradation.[2] Receptor desensitization by removal from the membrane and endocytosis is a feature shared by single-pass tyrosine kinase receptors and serpentine, heptahelical G-protein-coupled receptors (see Chapter 5).

It is not clear whether membrane-anchored growth factors (not bound to receptors), are also endocytosed, like their receptors, and if so how. The domain structure of some membrane-anchored growth factors and the high degree of conservation of their cytosolic domain have raised the question whether they may also be receptors, but there is no evidence to support such a function.

A process which, in most cases, modulates receptor signalling is phosphorylation. In the case of G-protein-coupled heptahelical receptors, the interaction with specific kinases is the first step in shutting off their action. In other cases, binding of a growth factor to a receptor triggers the intrinsic receptor kinase activity and leads to autophosphorylation. The important point is that the phosphates introduced in the receptor are essential for recognition and binding of other proteins, adaptors and transducers, which are often cytosolic protein kinases and phosphatases. Signalling triggered by growth factor–receptor interactions leads to a response which is often of global nature, such as growth, proliferation, and differentiation of cells. Growth factors affect the cell cycle and the cell death programmes, which determine the fate of the cell. Although, many processes vital for the cell are affected, the main target is the genome. The essence of cellular signalling is the transmission of signals from the surface of the cell to the nucleus and the subsequent expression of genes. Dysfunction of the regulatory mechanisms controlling these processes can cause malignant transformation of cells and other diseases.

I begin with signals that regulate cell growth and proliferation and describe the receptors that respond to these signals. This survey is by no means complete; only those growth factors and cytokines that we shall encounter again later are described.

Properties of signals

Although, principal features of receptor activation are preserved, different ligands and receptors account for the diversity of the biological effects.

Growth factors and hormones can engage in autocrine, juxtacrine, paracrine, and endocrine stimulation. If the receptor resides on the same cell where the ligand is expressed, the resulting cellular stimulation is autocrine; when the growth factor diffuses from the cell to neighbouring cells in the same organ, stimulation is paracrine, and juxtacrine stimulation is a mode of signalling reserved for those membrane-anchored growth factors which interact with receptors located on neighbouring cells. When a factor is transported through the bloodstream from the place of synthesis to other tissues equipped with receptors that recognize the factor, stimulation is endocrine, as in the case of hormones such as insulin.

Growth factors, cytokines, and hormones are, in large part, proteins. They are synthesized by the usual mechanisms of protein synthesis and transported through common routes of cellular protein traffic. Often inactive precursors are converted by limited proteolysis into active factors and shed by the cells.[3]

Processing of growth factors

Proteolytic processing is an ancient pathway, conserved from worms to humans. Processing of membrane-anchored growth factors is complex (cf. ref. 4). It usually involves cleavage of specific peptide bonds at the N- and C-termini of a large precursor. Cleavage and shedding of the ectodomains of plasma membrane proteins is carried out by transmembranous and soluble metalloproteinases, such as the matrix metalloproteinases (MMPs) and their relatives.[5] Serine proteinases have also been implicated.[6]

The first such 'sheddase' characterized was the tumour necrosis factor-α (TNF-α) converting enzyme (TACE).[10] TACE is a membrane-anchored proteinase and a member of the ADAM (*A d*isintegrin *a*nd *m*etalloproteinase) family of proteins that combine cell-surface adhesion and proteinase activity.[7–10]

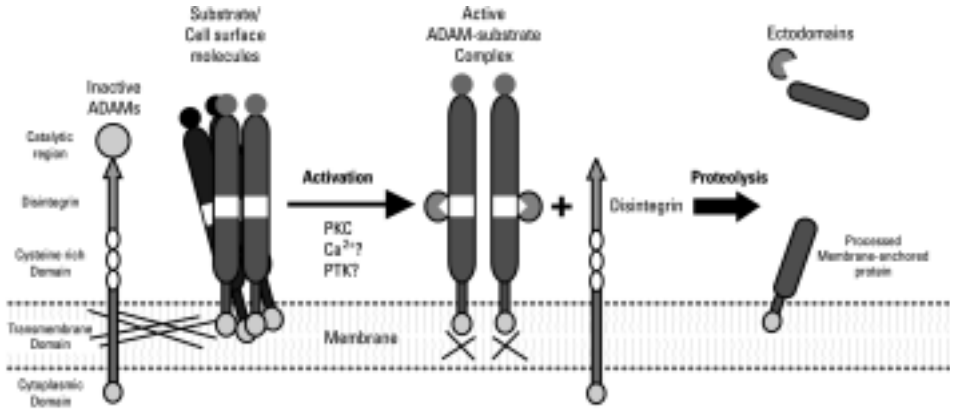

Fig. 1.2 A scheme of the activation of ADAMs and of the shedding process. The dimeric ADAM proteases and their substrates are anchored in the membrane, but are separated from each other. Upon activation (via protein kinases?), the protease is disengaged from disintegrin and associates with the substrate. Proteolysis takes place and the free, soluble ectodomains of membrane-anchored cell-surface proteins are shed from the membrane-anchored substrates and released. (Reproduced from ref. 3, with permission of the authors and *Science*.)

ADAMs, such as TACE, have a common domain organization (Fig. 1.2); they are zinc-dependent metalloproteinases, closely related to the MMP family. The action of the shedding enzymes is controlled by endogenous inhibitors, the TIMPs (tissue inhibitors of metalloproteinases).[11] The three-dimensional structure of TACE has been solved.[8] It is widely expressed and has several functions, among them the production of ligands for the epidermal growth factor (EGF) receptor. TACE is essential for epithelial development.[12] However, TACE and related metalloproteinases process not only growth factor ligands, they also cleave the ectodomains of receptors[13] and other membrane proteins.

Processing of membrane proteins by the ADAMs and other sheddases requires that both the membrane-anchored enzyme and its substrate are located on the same cell. The regulation of cleavage and the removal of the split products from the cell surface has been studied with angiotensin-converting enzyme (ACE), a typical sheddase.[14] These studies raised intriguing questions, of how the proteinases are kept apart from their substrates until shedding is triggered and, conversely, what brings the proteinase and its substrate together. Another question is why only ectodomains of certain transmembrane proteins, out of many such proteins on the cell surface, are targetted.

As for the first question: cytoskeletal interactions are apparently involved in keeping the proteinases and their transmembrane substrates apart, keeping them in distinct domains of the plasma membrane. See also: ref. 15. Upon activation, the cytoskeletal attachments seem to be loosened so that the proteinases and their substrates can approach each other and interact. How spatial restriction and compartmentalization of enzymes and substrates controls their action is of more general importance. We shall come back to that (Chapter 7).

Proteolytic processing of membrane-anchored proteins is an important event. TNF-α, TGF-α, EGF, heparin-binding EGF-like growth factor (HB-EGF), the Kit ligand, and other growth factors must all be processed to become functional. Processing has other

important consequences: cleavage of adhesive molecules profoundly affects cell–cell adhesions and cell–cell interactions in mammary epithelial cells; dysfunction of processing may cause cancer.[16] Processing of the Notch receptor plays a central role, not only in cell adhesion but also in neurogenesis in vertebrates and Drosophila.[17] In Drosophila Notch controls cell–cell interactions that are involved in the diversification and differentiation of cells during development. The Notch receptor participates in cell development and in the selection of cell lineages. It is activated by protein ligands, localized on the surface of neighbouring cells, rather than by soluble ligands. This kind of receptor, interacting with adhesive ligands, may also participate in pathfinding in the nervous system. Finally, shedding can also control cell death by processing ligands for death receptors (see Chapter 13).[18]

Many growth factors that become soluble after having been processed are not shed and remain anchored to the membrane. Therefore, the distinction between membrane-anchored[19] and diffusible growth factors is somewhat ambiguous. In malignant transformed cells, diffusible factors may be retained in the membrane of the same cell where they are synthesized and processed. This can stimulate the cell in an autocrine fashion with deleterious effects.

A few specific details on the processing of growth factors and hormones follow.

Processing of pro-TGF-α

TGF-α is a growth factor.[20] Its processing is of interest. Pro-TGF-α is a 160-amino-acid protein which is heterogeneously N- and O-glycosylated.[21] The whole extracellular ectodomain of pro- TGF-α, with the sugars, is cleaved off and secreted.[22]

Mutations have identified the C-terminal valine, in the highly conserved cytoplasmic region of pro-TGF-α, as the critical determinant for cleavage. Substitutions elsewhere throughout the cytoplasmic region have no effect on cleavage, as long as valine remains the C-terminal amino acid. Replacement of this residue abolishes cleavage, and even conservative substitutions of this valine markedly impair processing, suggesting a highly specific recognition event. The simplicity of a single residue, a C-terminal valine, as the pro-TGF-α cleavage signal is striking. How this C-terminal valine determines cleavage is a matter of speculation.[23]

The colony-stimulating factors

The granulocyte–macrophage colony-stimulating factors (GM-CSFs) are cytokines (Chapter 6). The gene for CSF-1 encodes a 554-amino-acid precursor protein. A huge portion is removed and only a small region of 36 amino acids is retained. The final macrophage colony-stimulating factor is a soluble, heterogeneous proteoglycan.[24,25]

TNF-α and TNF-β

Two kinds of tumour necrosis factors exist: TNF-α and -β. They are either membrane bound or soluble. TNF-α is identical with cachectin,[26]. It is produced in monocytes and macrophages; TNF-β is synthesized mainly in lymphocytes. Only TNF-α is processed and cleaved from a membrane-bound glycoprotein.[27] The processed, mature human TNF-α is a 145–157-amino-acid peptide chain, depending on the cleavage site. The chains associate, forming a soluble trimer.

Vascular endothelial growth factors

Vascular endothelial growth factors (VEGFs) are cytokines which increase vascular permeability and promote angiogenesis and blood vessel growth during embryogenesis[28]

(a second family of vascular growth factors are the angiopoietins). VEGFs also promote the formation of lymphatic vessels.[29] One of the VEGFs, VEGF-C, is synthesized as a precursor and must be processed for activation. Apart from VEGF, growth factors such as fibroblast growth factor (FGF) and TGF-α also promote angiogenesis. Angiogenic factors have an important role in the growth of cancers and in metastasis.[30] Therefore there is great interest in the development of blockers of VEGFs to slow down the growth of cancer.

Processing of pro-insulin

Inclusion of insulin in the group of growth factors may be disputed. Processing as exemplified above is a post-synthetic process, whereas processing of pro-insulin is part of the synthesis of insulin, taking place at the site of synthesis, the β-cells of the pancreas. It is the final step in the formation of an active hormone. But since insulin processing is known in great detail, mainly thanks to the work of Donald Steiner and his laboratory,[31] and since insulin signalling is discussed later (Chapter 8), insulin processing will be included here.

The gene for insulin is located on the short arm of chromosome 11. Expression of the insulin gene yields a precursor protein (preproinsulin), of 104–109 amino acids, depending on the animal species, with a 24-amino-acid signal peptide. After removal of the signal peptide in the endoplasmic reticulum, proinsulin is formed. Proinsulin resembles the insulin-like growth factors, IGF-1 and IGF-2 (7 kDa proteins), and the female hormone, relaxin, which are larger than the finally processed insulin. The next step involves generation of inter- and intrachain disulphide bridges, facilitated by the removal of the C-peptide. The separate A and B chains of mature insulin are formed and held together by interchain disulphide bridges (Fig. 1.3).

Processing is carried out by a family of well-characterized proteases. Conversion takes place in secretory granules and the excised C peptide is secreted together with mature insulin into the bloodstream. Peptide C is thought to link the A and B chains in the precursor in a way that facilitates folding and efficient formation of the interchain disulphide bonds. No receptors for the C peptide have been found, and all the available evidence suggests that the C peptide does not interact with a receptor, although the C peptide was recently claimed to have biological activity.[33]

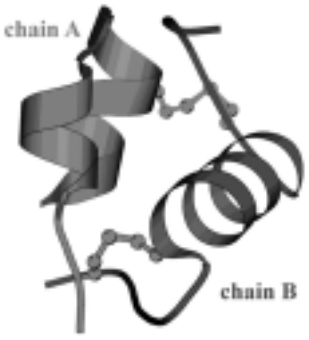

Fig. 1.3 Parts of the disulphide-linked A and B chains of bovine insulin. (Reconstructed from data[32] deposited in the Brookhaven protein databank, with permission of Professor D. L. D. Caspar and the Biophysical Journal.)

Receptors for growth factors and their ligands

Growth-factor signalling is mainly mediated through receptors with tyrosine kinase activity (RTKs) (Table 1.1). Activation of these receptors involves dimerization and autophosphorylation on tyrosyl residues. The phosphotyrosyls then become docking sites for intracellular regulatory proteins, linkers, which are instrumental in transmitting the signal (Fig. 1.4).[34]

Table 1.1 The properties of some growth factors and their cognate receptors

Growth factor	Properties	Receptor	Properties
Epidermal growth factor (EGF)	EGF-unit with 6 conserved cysteines	EGF receptor	Single pass Tyrosine kinase receptor
Transforming growth factor-α, (TGF-α)	40% homologous with EGF	EGF-like receptor	Dimerization Autophosphorylation
Neuregulin	Neuregulin signalling is involved in the development of the heart and the peripheral nervous system[48]	ErB2, ErB3, ErB4	Truncated EGF receptors
Platelet-derived growth factor (PDGF)	Homo- and heterodimeric AA, BB, AB forms	PDGF-receptor-α PDGF-receptor-β	Homo- and heterodimeric Tyrosine kinases
VEGF	Vascular endothelial growth factor expressed in endothelial cells	VEGFR	PDGF-like receptors
MCSF-1	Macrophage colony factor	MCSFR	" "
Acidic and basic fibroblast growth factors (FGFs)	Tissue-specific functions FGFs bind to heparan-sulphate glycosaminoglycans	FGFRs	Homo and heterodimeric tyrosine kinases
Keratinocyte growth factors (KGFs)	Wound healing	FGF-like receptor	" "
Kit/KL: mast cell growth factor, stem cell or steel factor	Two heterogeneously glycosylated forms. KL-1, KL-2. Role in haemalopoiesis and in the development of cell lineages	c-Kit receptor	Receptor tyrosine kinase
Insulin	Circulating hormone, formed and secreted from the β-cells of the pancreas	Tyrosine kinase receptor with constitutive oligomeric structure	Heterodimer consisting of α- and β-disulphide-linked subunits
	Blood sugar regulation		Autophosphorylation Forms a tetramer on ligand binding
Insulin-like factor	Regulates metabolism and longevity in *Caenorhabditis elagans*	Insulin-like receptors, DAF-2 in *Caenorhabditis elegans* and *Drosophila*	Tyrosine kinase domains
HGF, SF hepatocyte growth factor, scatter factor	Dissociates sheets of epithelial cells and promotes cell mobility, stimulates cell proliferation and induces cell polarity. Mutations cause cancer	Insulin-like receptors	Tyrosine kinases
HGF-like factor	Factors implicated in axonal pathfinding	Axl receptors	Tyrosine kinases, related to the insulin receptor
Ephrins	Ephrins may be involved in cell–cell signalling	Eph-like receptors	Tyrosine kinases

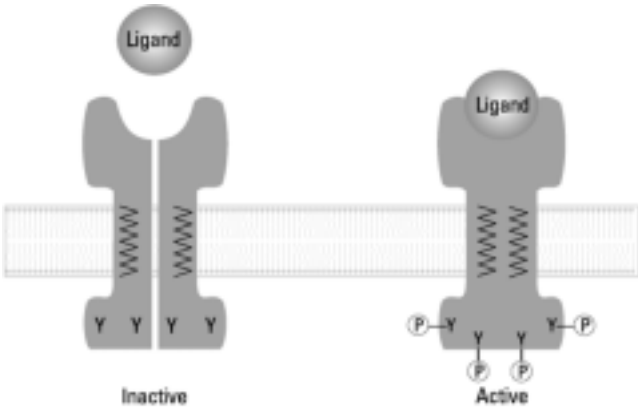

Fig. 1.4 Receptor dimerization and autophosphorylation of tyrosines (Y) on ligand binding. (The molecular basis of this mode of receptor activation is discussed in Chapter 2).

The EGF family of growth factors and the cognate receptors

EGF was the first growth factor to be identified. This was achieved by Stanley Cohen. see ref. 35. It is the prototype of a heterogeneous family of growth-promoting proteins, which all share one or more EGF-like structural units.

The EGF-unit is defined by six conserved cysteines in a stretch of 35–40 amino acids: $CX_7CX_{3-5}CX_{10-12}CXCX_5GXRC$, where C is cysteine; G, glycine; R, arginine; and X any other amino acid. The six cysteines form three disulphide bonds, C1–C3, C2–C4, and C5–C6. This motif is conserved in all EGF-like growth factors. Some of them contain calcium-binding motifs (for a review see ref. 36).

A variety of quite different factors belong to the family of EGF-like growth factors, among them TGF-α. TGF-α shares 44% and 33% homology with human and mouse EGF, respectively.[37] Whereas TGF-α binds to a typical receptor tyrosine kinase, TGF-β binds to receptors which are serine–threonine-specific kinases (Chapter 6). Other growth factors with EGF structural units are the heparin-binding EGF-like growth factor (HB-EGF)[38] and the vaccinia virus growth factor (VGF). They all bind to EGF receptors. The EGF unit of HB-EGF contains a N-terminal extension, rich in basic amino acids, that is responsible for binding heparin. Many different growth factors bind to one and the same EGF receptor, although the amino-acid sequence identity between these factors and EGF is less than 35%. This points to plasticity of the receptor interaction surface. Furthermore, each of these different factors elicits a distinct biological response, depending on the cell type (e.g. epithelial or mesenchymal cells). The cell-specific biological response implicates the existence of unique signal-transducing pathways in each differentiated cell. In some cases, the cell provides a different adaptor which directs the signal to a different target.

As EGF is a prototypical growth factor, EGF receptors (EGFRs) are prototypes of receptor tyrosine kinases (RTKs). Homologues of the EGFR exist in Drosophila (DER)[39] and in Caenorhabditis elegans (LET-23).[40]

The non-activated EGF receptor is a single-pass transmembrane protein of 170 kDa (Fig. 1.5).

All receptors of the EGF family have two cysteine-rich clusters in the extracellular region and a tyrosine kinase domain in the cytoplasmic part. Autophosphorylation sites are in the C-terminal regions of the cytoplasmic domain. On activation, five tyrosines are

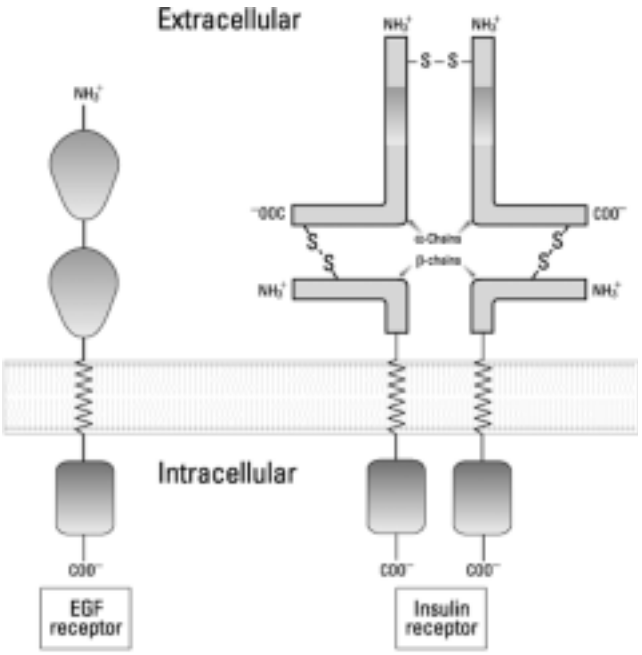

Fig. 1.5 The EGF receptor is compared with the insulin receptor, which is a constitutive heterodimer, consisting of α- and β-chains.

autophosphorylated. With the help of these specific phosphotyrosyls, coupling partners are recruited with amazing precision.[41]

There exists a family of truncated, EGF-like receptors, (ErB2, ErB3, ErB4), which regulate the neu gene (neu is an oncogene derived from the DNA of a rodent neuroblastoma, it is also known as *ERBB2* or *HER2*).

Platelet-derived growth factors and their receptors

Platelet-derived growth factors (PDGFs) exist in three different dimeric compositions, containing disulphide-linked A-, or B-, or A- and B-chains (AA, BB, or heterodimeric AB). The composition of the ligand dimers matches the composition of the receptor dimers to which they bind (Fig. 1.6). Therefore, a specific response of cells to PDGF depends on the composition of the ligand dimer which is recognized by a fitting receptor dimer. One of the aims of research in this active field is to identify different signalling pathways for each receptor–ligand combination.[42] Platelet-derived growth factor receptors, PDGFR-α and PDGFR-β, have quite similar primary sequences and domain structures, but are expressed in different cells. PDGFRs have extracellular immunoglobulin-like domains and a cytoplasmic domain with a large tyrosine kinase insert which presents the phosphotyrosines for coupling to signalling molecules. Upon binding a matching PDGF, the receptors form non-covalent homo- and heterodimers.

PDGF-like receptors are VEGFR, the receptor for the vascular endothelial cell growth factor, which is expressed only in endothelial cells, and the receptor for the MCSF-1, the macrophage colony-stimulating factor-1. PDGFRs have, like EGFRs, multiple autophosphorylation sites, each of which is, again, specific for a particular recognition domain of a coupling partner. For example, phosphorylation of tyrosine 1021 in the receptor tail of PDGFR-β is responsible for binding to phospholipase C (PLC-γ1). A mutated receptor,

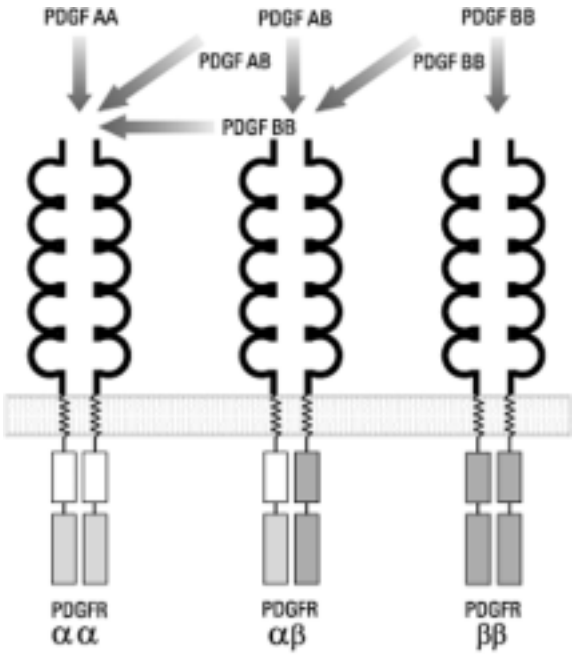

Fig. 1.6 Homo- and heterodimerization of the PDGFR-α and -β chains is matched by the assembly of the homo- and heterodimeric ligands, PDGF A and B. (From Lemmon and Schlessinger,[43] with permission of the authors and Trends Biochem. Sci.)

where Tyr1021 was replaced by Phe, could not activate the PLC pathway, but could still activate other signalling paths.[44,45] PLC-γ is a common receptor target. It couples with high affinity to the EGF receptor. This interaction is also mediated by a phosphotyrosyl residue (Y992 of the EGFR). The consequences in both cases are the same, phosphorylation and activation of PLC-γ[46,47] and formation of the second messenger, IP_3 inositoltrisphosphate. Other enzymes which are downstream mediators of growth factor receptor signals are the phosphatidylinositol-3-OH kinase[48] which, together with PLC-γ, propagates the mitogenic signal of the PDGF receptors.

Among proteins addressed by growth factor receptors are also Raf kinase (and other cytosolic kinases) and also monomeric GTPases, such as Ras. They play a central role in the regulation of growth factor signalling. (These pathways are discussed in Chapter 4). Each of the different partners recruited by the activated receptor initiates a different signalling pathway, making possible a great variety of cellular responses.[49] I shall come back to this when explaining how these proteins recognize each other and build cellular signal transmission chains over which the signal travels from the receptor on the membrane to the genes in the nucleus. At this point, I only wish to emphasize the coupling versatility of growth-factor RTKs.

Fibroblast growth factors and their receptors

At present, seven members of the fibroblast growth factor (FGF) family are known. Among them are the acidic and basic FGFs, aFGF and bFGF,[50] the keratinocyte growth factor (KGF),[51] which is involved in wound healing in the skin, and the angiogenic factors.[52,53]

In common with PDGFRs, the FGF receptors are single-pass polypeptide chains with a membrane-spanning domain, an extracellular region with three immunoglobulin-like

domains, and a cytoplasmic region with a catalytic tyrosine kinase domain. A short insert splits the kinase domain into two nearly equal halves. Differential splicing in the exons for the extracellular region of fibroblast growth factor receptor 1 generates receptor variants with different ligand-binding specificities.[54] FGFR variants with different ligand-binding specificities also arise from different exons in the FGFR1 and FGFR2 genes, expressed in a tissue-specific manner (exons are those parts of the genome that are expressed and transcribed into proteins).[55]

FGF receptor isoforms, FGFR1, FGFR2, and FGFR3, form both homodimeric and heterodimeric receptor species. The multiplicity of FGF receptors[56,57] explains the selective, individual responsiveness of cells and tissues to either acid or basic FGFs.

Binding of FGFs to their receptors has the familiar consequences: activation of the receptor tyrosine kinase and autophosphorylation (probably intermolecular) of the dimerized receptor.[58] Binding of FGFs to their cognate receptors is facilitated by heparan sulphate-type glycosaminoglycans.[59] These molecules belong to the ground substance, the glycocalix surrounding cells. They are made up of disaccharide units, containing a derivative of an amino sugar, either glucosamine or galactosamine with N- and O-linked, negatively charged sulphate and N-acetyl groups.[60] Crystal structures of complexes of bFGF with tetra- and hexasaccharides, derived from heparin, showed that no significant conformational change in bFGF occurs on association with saccharides, supporting the notion that glycosaminoglycans in the extracellular matrix only help to bring FGF to the membrane where the receptor is, but have no function in signalling (the same has been assumed in the case of HB-EGF). Thus, the idea was, until recently, that heparan sulphate proteoglycans, HSPGs, help to bind and protect ligands and concentrate them at the cell surface, but have no specific functions. However, there are proteoglycans, functioning as co-receptors, which participate in growth-factor signalling. One of them is a membrane-bound core protein, syndecan. Syndecans are transmembrane, heparan sulphate proteoglycans which interact with extracellular ligands and may facilitate the formation of receptor–ligand complexes.[61] Proteolysis of syndecan converts the syndecan co-receptor to a potent inhibitor of FGF-2.[62] New information from genetic studies in Drosophila and mice are beginning to reveal unique functions of HSPGs in specific signalling pathways involved in cell differentiation and morphogenesis.[63] The study of mutant phenotypes of either the enzymes synthesizing the heparan sulphate side-chains of the core proteins or synthesizing the core proteins themselves have indicated that the HSPGs are critical for signalling, specifically for the interactions of extracellular ligands with their signal-transducing receptors in the cell membrane.

Like other growth factors, FGF has distinct and different effects in different cell types. For example, in PC12 cells FGF promotes neurite outgrowth, in NIH3T3 cells it regulates cell proliferation and mitogenesis, and in Xenopus embryos it controls the formation of the mesoderm.

Interactions between the activated, autophosphorylated FGFRs and their coupling partners are as specific as EGFR and PDGFR interactions. For example, when Y766 is replaced by F (phenylalanine) the mutant receptor can neither associate with nor phosphorylate PLC-γ, and consequently some of the characteristic FGF responses are missing, such as an increase in intracellular calcium due to formation of second messengers (such as IP3), which is promoted by PLC-γ. But cells expressing this FGFR mutant, Y766F, can still respond to fibroblast growth factors with stimulation of cellular proliferation, suggesting that PLC-γ is not involved in this signalling pathway.[64] From this and

similar evidence one can conclude that, by engaging different transmitters, different signalling routes can be triggered by the same receptor, giving rise to different cellular responses.

The 'KIT' receptor and its ligand

Since later chapters deal with the development of differentiated cell lineages (Chapters 6 and 14), the receptor c-KIT, and its ligand (KL), are introduced here as an example of RTKs participating in the control of haematopoiesis. The c-KIT receptor is encoded by the proto-oncogene of a feline sarcoma retrovirus. It is involved in the development of haematopoietic stem cells and of melanocytes. Autophosphorylation upon ligand binding initiates signal transduction and recruitment of cytosolic signal transducers.[65] Signal transmission is regulated by the receptor tyrosine kinase activity of the c-KIT receptor.[66,67]

KL or 'KIT' is also known as the 'mast cell growth factor', or 'stem cell' or 'steel' factor.[68] KL and the cognate receptors are transcribed from genes in loci essential for mouse haematopoiesis.[69] Alternative splicing yields two KL forms, KL-1 (273 amino acids) and KL-2 (245 amino acids).[70,71] Both forms are heterogeneously glycosylated, giving rise to various cell-bound and soluble forms. On splicing, the shorter KL-2 form loses its main proteolytic processing site and remains bound to membranes.

Receptors for neurotrophins

The neurotrophins and their receptors are included in this introductory chapter on ligands and receptors participating in cellular signalling. References are provided so that the interested reader has access to the literature, to give him or her a more comprehensive view of this interesting field, since signalling in the neuronal system is not treated explicitly in this book.

Currently, there are six members of the neurotrophin family, including nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), glial-cell-line-derived neurotrophic factor, (GDNF),[72] and 'neurturin', which is structurally related to GDNF,[73] as well as several neurotrophins, NT-3, -4, and -5.

Three genes for vertebrate neurotrophin RTKs have been cloned. The three RTKs, A, B, and C, are highly selective with respect to the ligands they accept: the ligand for RTKA is NGF and BDNF, and the ligands for RTKB are the neurotrophins, NT-4/5, whereas NT-3 is bound exclusively to RTKC (Table 1.2).[74–76]

NGF was discovered by Rita Levi Montalcini in Victor Hamburger's laboratory at the Washington University in St Louis. It was also the first neurotrophin of which the structure (Fig. 1.7) was solved (reviewed in ref. 77,78). Originally it was thought that the 'cystine-knot' motif[79] in the ligand is important for recognition and for discrimination between different receptor tyrosine kinases. But this can no longer be upheld: ligands with a cystine-knot motif bind to structurally and functionally dissimilar receptors. The cystine-knot motif is a core structure, found also in transforming growth factor-β (TGF-β), PDGF, and human chorionic gonadotrophin (hCG). Whatever the role of the cystine-knot motif, it is probably not a structural motif, determining receptor–ligand interactions because, if that were the case, one would expect a matching motif in the diverse group of receptors which all accept ligands with a 'cystine-knot motif'. But such a motif has not been found.

Table 1.2 Summary of the neurotrophins and their receptors

Growth factor	Properties	Receptor	Properties
Nerve growth factor (NGF)	Cystine knot motif Promotes cell death in the nervous system, when bound to $p75^{NGFR}$	Two types of receptor, co-receptor? NGFR-α,β	$p75^{NGFR}$, have no tyrosine kinase activity. They are cytokine-type receptors like the TNF-receptor
NT-3, -4, and -5	Several neurotrophic factors, including NGF Support survival of neurons	NTFR	Tyrosine kinase receptors, 140 kDa, RTKs A, B, C
Brain derived neurotrophic factors (BDNF)	Support survival of neurons	BDNFR	Tyrosine kinase with large cytoplasmic region. Several subtypes
Glial-cell-line derived neurotrophic factor (GDNF)	Several neutrophic factors including NGF, ciliar neurotrophic factor, and neurturin, related to GDNF. Supports survival of glial cell lines	GDNFR-α and RET receptor	RET receptor is a tyrosine kinase. GDNFR-α is a co-receptor which is required for activation of the RET receptor

Two different classes of transmembrane receptors are involved in signal transduction by the neurotrophins; one is an RTK, whereas the other, $p75^{NGFR}$, has no tyrosine kinase activity. $p75^{NGFR}$, has been classified as a member of the TNF-receptor family,[80] although the intracellular part of $p75^{NGFR}$ shows no homology to any known protein. The p75 NGF receptor is widely expressed in neuronal and also in non-neuronal cells and tissues, but its function is still controversial. The original assumption that NGF is a trophic factor for sympathetic neurons must be qualified, because how NGF functions depends on the kind of receptor to which it binds. NGF bound to $p75^{NGFR}$ receptors actually promotes cell death in the nervous system,[81] because $p75^{NGFR}$ is, like the TNF receptor, a 'death receptor' (see Chapter 13). On the other hand, binding of NGF to RTK receptors supports survival of neurons. This class of neurotrophin receptors comprises typical 140 kDa tyrosine kinases, with large cytoplasmic regions. Binding of neurotrophins leads to receptor dimerization, autophosphorylation, and activation.

Interaction of neurotrophin RTKs with $p75^{NGFR}$

The fact that the NGF-binding RTK and the $p75^{NGF}$ receptor are co-precipitated by antibodies in cell extracts raised the possibility that both types of receptors may interact with each other.[82–84] Some kind of a co-receptor function of $p75^{NGFR}$ for activation of the neurotrophin p140 RTKs, would be reminiscent of the co-receptor function of GDNFR-α in the activation of RET, the RTK which mediates signalling of GDNF, the glial-cell-line-derived neurotrophic factor. The co-receptor, GDNFR-α, has no cytosolic domain and therefore is incapable of signalling. For signalling, it needs the RET eceptor.[85,86] The co-receptor GDNFR-α is found in all cells and tissues that are GDNF responsive. Expression of GDNFR-α seems to be controlled developmentally. Several isoforms of GDNFR-α are expressed in different parts of the brain. A homologue of GDNFR-α facilitates binding of another neurotrophic factor, ciliar NTF, to its receptor.

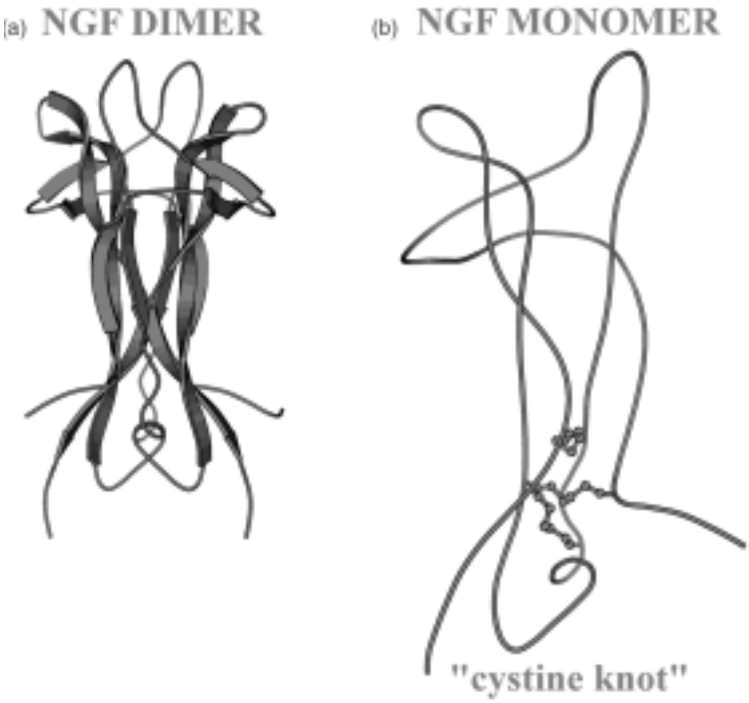

Fig. 1.7 (a) The crystallographic structure of 7S dimeric mouse NGF. (Reproduced with permission of Professor Thomas Blundell and Structure from ref. 78, using data from protein data banks). The dimeric NGF molecule is rather asymmetric and has a flat, elongated shape, a feature for which the presence of two pairs of antiparallel β-strands in each NGF subunit are mainly responsible. These β-strands are linked at the top to three short, highly flexible loops. The flexibility of the loops accounts for the fact that NGF assumes different structures in different crystal preparations. At the other end of the NGF molecule are three neighbouring disulphide bridges with a characteristic topology. Two of these disulphide bridges (Cys58–Cys108 and Cys68–Cys110) form a ring-like structure through which the third disulphide bridge (Cys15–Cys80) passes and forms a tightly packed 'cystine knot' motif. NGF was the first protein shown to have a cystine knot structure. The 'cystine knot' motif of the NGF monomer is reconstructed and shown in (b). (b) The 'cystine knot' arrangement allows the two pairs of β-strands from each subunit of NGF to pack against each other, generating an extensive interface in the dimer. The fact that mainly aromatic amino acids, capable of hydrophobic interactions, are at the interface explains the tight association of the subunits.[86] The preservation of these structural features in neurotrophins explains why they can readily form mixed heterodimers with each other.

It was suggested that GDNF associates first with the co-receptor, GDNFR-α, and the GDNF–GDNFR-α ligand–co-receptor complex is then presented to the RET receptor. RET receptors are tyrosine kinases. The RET receptor is the product of the c-ret proto-oncogene. (The name was given to a transforming human oncogene, ret, that originated from a T cell lymphoma DNA). A single point mutation in the tyrosine kinase domain is a gain-of-function mutation and results in deregulated activation of the RET receptor

tyrosine kinase, often found in humans with a predisposition to endocrine cancers, such as thyroid cancers. Loss-of-function mutations were found in Hirschsprung's disease, a developmental disorder with defective intestinal innervation. Moreover, somatic rearrangements of the *ret* proto-oncogene have been found in about 60% of papillary thyroid cancers in children exposed to radiation in the Chernobyl catastrophe (Chapter 15).

Hormone receptors and orphan receptors

The insulin receptor will be discussed here as a prototype of a receptor with tyrosine kinase activity which transmits a hormonal signal. The other major classes of receptors which transmit hormonal signals are the heptahelical, G-protein-coupled receptors and the nuclear receptors for steroid and non-steroid hormones (see Fig. 1.1); they are discussed later. The receptor for the peptide hormone insulin is one of the most extensively studied RTKs (receptors with tyrosine kinase activity).

RTKs that interact with peptide hormones

The insulin receptor

The insulin receptor is the prototype of a tyrosine kinase receptor with a constitutive oligomeric structure. The signalling form is a $\alpha 2/\beta 2$ tetramer, but the unit structure is a heterodimer, consisting of α- and β-subunits. But, in contrast to the homo-and heterodimers of other RTK growth factor receptors, the formation of the insulin receptor oligomer is constitutive and not dependent on ligand binding (see Fig. 1.5).

The α-chain and the β-chain of the receptor are linked by disulphide bridges. The α-chains are exclusively extracellular, whereas the β-chains have extracellular-, transmembrane-, and cytosolic domains. The extracellular domain of the α-subunit contains the ligand-binding site. Binding of insulin leads to autophosphorylation of the β-chains. Three groups of phosphotyrosyls are crucial for signalling. They are in the cytosolic part of the β-chains, in their carboxy-terminal tail, and in a region close to the membrane. When Y960, located close to the membrane, was replaced, the mutant receptor was inactive, although insulin could still stimulate the receptor tyrosine kinase activity. This is another impressive example of the specific role of a single tyrosyl phosphate for receptor signalling (see refs 89–92).

A central question is, of course, the relationship of the tyrosine kinase activity of the receptor to the action of the ligand, the hormone, insulin.[93] This will be discussed in Chapter 8.

Receptors that share similarities with the insulin receptor

Homologues of the mammalian insulin receptor, IR, are the DAF-2 receptor in Caenorhabditis elegans[94] and the IR-like receptor in Drosophila.[95] The DAF-2 receptor shares 35% of its amino-acid sequence with the human insulin receptor and 34% with the insulin-like growth factor receptor-1. The tyrosine kinase domain of the DAF-2 receptor is 70% similar and 50% identical to the tyrosine kinase domain of the human insulin receptor. A ligand for DAF-2 has not yet been identified, but an insulin-like peptide is anticipated. Since a typical insulin receptor substrate, like IRS-1 or IRS-2, has not been found in C. elegans, it is assumed that a COOH-terminal extension of the DAF-2 receptor serves as a built-in receptor substrate, which when phosphorylated, helps to recruit signalling proteins.

The close relationship of the human insulin receptor with the IR-like receptors in C. elegans and Drosophila indicates a common origin of these receptors, going back about 700 million years, to a time before invertebrates and mammals diverged. The fascinating similarities of signalling through the DAF receptor in nematodes and the insulin receptor in humans are discussed in Chapter 8.

The hepatocyte growth factor (HGF) receptor also belongs to the group of insulin-like receptors. The ligand for this receptor, HGF, is identical with the scatter factor, SF. This factor has been named 'scatter factor' because it dissociates sheets of epithelial cells and stimulates their motility. Binding of the factor activates the receptor tyrosine kinase and leads to autophosphorylation and association with a variety of signalling proteins, opening the door to different signalling pathways.

HGF is essential for the development of epithelia.[96,97] It stimulates cell proliferation, motility, and induction of cell polarity. Genetic knock-out of either the ligand or the HGF-receptor is lethal for the embryo. It is not surprising, therefore, that deregulation and overexpression of the *met* gene, which encodes the HGF receptor, can cause invasive growth of epithelial cells, contributing to the metastatic properties of cancer cells. Germ-line and somatic mutations of the *met* gene were found in patients with kidney carcinoma. The met gene was first identified in cells treated with the mutagen N-methyl-N-nitrosoguanidine.

A receptor more distantly related to this group of receptors is the bacterial (Escherichia coli) aspartate receptor, which promotes movement of the bacteria and chemotaxis in response to the attractant, aspartate. The aspartate receptor is not a tyrosine kinase (see also Chapter 2).

RTKs in search of a ligand

Finally, one should keep in mind that there exists a large family of RTKs, the so called Eph-like receptor tyrosine kinases, for which the ligands (the 'Ephs') are not well defined (*eph* is a gene, named from an erythropoietin-producing hepatocellular carcinoma cell line). At least seven genes have been identified which code for these receptors, and more can be expected (cf. ref. 98). Eph-like receptor tyrosine kinases interact with unusual membrane-associated and membrane-spanning molecules, the 'ephrins', which appear to be involved in cell–cell signalling.[99] An Eph-like receptor, the Axl receptor tyrosine kinase, is related to the insulin receptor and has been implicated in axonal pathfinding.[100]

The G-protein-coupled receptors

The G-protein-coupled heptahelical receptors are the largest transmembrane receptor class. They may take up 2% of the genome. Altogether, there may be thousands of different receptor molecules of this type that all transmit their signals through a heterotrimeric GTP-binding protein. These receptors are dealt with in Chapter 5.

The receptors for TGF-β and the cytokine receptors

The receptors for TGF-β and cytokines either are Ser/Thr kinases or have no kinase activities. They are considered in Chapter 6.

Conclusion

In this chapter growth factors and receptors have been surveyed and processing of ligands and receptors has been discussed. The first step in cellular signalling, the recognition of extracellular signals by membrane-bound sensors and the autophosphorylation of the

tyrosine kinase receptor upon ligand binding and activation, has been introduced, and it has been shown that the introduction of a single phosphate group, esterified with the OH-group of a tyrosine on a strategic position on the recognition surface of the receptor, determines which partner is chosen for coupling. The recruitment of the partner is specific. This event may determine the route the signal travels. The structural basis for protein–protein recognition will be discussed in Chapter 3.

In Chapter 2, I shall explain a general principle of activation of membrane single-pass tyrosine kinase receptors—receptor dimerization.

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